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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: BIOSENSORS WHICH UTILIZE CHARGE NEUTRAL CONJUGATED POLYMERS (54) Titre: BIOCAPTEURS UTILISANT DES POLYMERES CONJUGUES A CHARGE NEUTRE (57) Abstract <p>This invention encompasses charge neutral conjugated polymer or copolymers which have a functional group for binding a biomolecule probe; electrodes and array of electrodes in electrical contact with such polymers and wherein a biomolecule probe is covalently lined to the polymer. The invention includes biosensors which utilize the conjugated polymer coated electrodes wherein the binding to the biomolecule probe is detected by electrical means such as AC impedance.</p> (57) Abrégé <p>La présente invention concerne un polymère conjugué à charge neutre ou des copolymères qui possèdent un groupe fonctionnel permettant la liaison d'une sonde biomoléculaire. Cette invention concerne aussi des électrodes et un réseau d'électrodes en contact électrique avec ces polymères, et une sonde biomoléculaire est liée de façon covalente au polymère. Cette invention comprend des biocapteurs qui utilisent les électrodes revêtues du polymère conjugué, et la liaison de la sonde biomoléculaire est détectée par une composante électrique telle qu'une impédance relative au courant alternatif.</p>		

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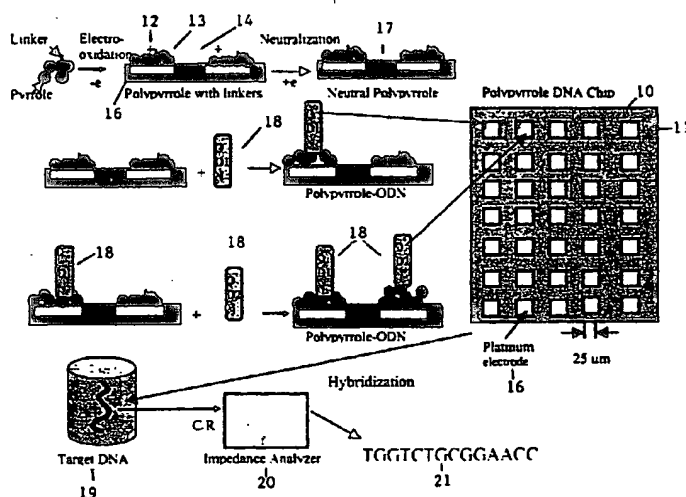
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(54) Title: BIOSENSORS WHICH UTILIZE CHARGE NEUTRAL CONJUGATED POLYMERS



(57) Abstract: This invention encompasses charge neutral conjugated polymer or copolymers which have a functional group for binding a biomolecule probe; electrodes and array of electrodes in electrical contact with such polymers and wherein a biomolecule probe is covalently lined to the polymer. The invention includes biosensors which utilize the conjugated polymer coated electrodes wherein the binding to the biomolecule probe is detected by electrical means such as AC impedance.



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Description

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**BIOSENSORS WHICH UTILIZE CHARGE NEUTRAL
CONJUGATED POLYMERS**

This invention claims the priority of provisional application Serial No. 60/138,437 filed June 10, 1999.

FIELD OF THE INVENTION

The invention is in the field of arrays of sensing electrodes on a chip for conducting analysis of biological substances such as DNA.

BACKGROUND OF THE INVENTION

A device for biomolecule detection is generally comprised of supporting matrix for probe molecule attachment or entrapment, a sensing probe located on/in the supporting matrix. When exposed to a complementary biomolecule target (or analyte), the biosensing device produces detectable change in radioactive, optical, or electrical signal to confirm the existence of a specific biomolecule target. In general, the biomolecule target to be detected needs to be labeled with a marker (or reporter) such as ^{32}P , fluorescent dye, or redox, depending on whether the detection means is autoradiography, fluorescent microscope or electric tools.

An alternative biosensing device includes a second reporting molecule. The second reporting molecule is introduced after the probe molecule has interacted with its complementary biomolecule target. Like the probe molecule, the second reporting molecule also interacts with the biomolecule target by either binding to the target or forming a complex.

Lavache, et al Analytical Biochemistry 258, 188-194 (1998) describes an

5 oligonucleotide array constructed on a silicon chip having a matrix of addressable
microelectrodes. Each electrode is coated with polypyrrole copolymer where some
10 of the pyrroles in the copolymer have an oligonucleotide bound to the pyrrole. The
polymers are made by electrochemical techniques. This copolymer is deposited on
microelectrodes. Hepatitis C genotypes were detected by hybridization of the probe
15 DNA on the electrode to test sample DNA which was PCR amplified to contain a
fluorescent marker group.

WO 95/29199 describes functionalized polypyrrole copolymers where the
20 functional groups are designed to bind biological molecules such as DNA or
polypeptides.

15 US Patent 5,837,859 assigned to Cis Bio International describes the
preparation of electrically conductive pyrrole/nucleotide/derivatized/pyrrole
copolymers useful for nucleic acid synthesis, sequencing and hybridization. The
copolymers are produced electrochemically and coated on microelectrodes for DNA
30 analysis.

20 US Patent 5,202,261 describes conductive sensors and their use in diagnostic
assays.

US Patent 5,403,451 describes the detecting of a target analyte with
35 conductive
polymer coupled with periodic alternating voltage.

25 In a typical prior art, the target DNA is usually labeled with a marker (or
reporter)
45 such as ^{32}P , fluorescent dye, or redox. When the labeled target is exposed to its
complimentary probe on the conductive polymer or copolymer, a radioactive signal,
50 or fluorescence, or electric signal is detected. Generally, fluorescent or redox labeling

5 is preferred due to the stringent experiment conditions required for radioactive
labeling. However fluorescent dyes in the vicinity of conductive polymers or
10 copolymers are subject to signal quenching. On the other hand, conductive polymers
or copolymers contribute to significant background noise when used for redox labeled
target detection.

10 SUMMARY OF THE INVENTION

15 It is an object of the present invention to eliminate the signal quenching from
conductive polymers when used as supporting matrix for probe attachment or
20 entrapment for biomolecule detection and a biosensing device to carry-out such
detection.

25 It is another object of the present invention to reduce the detection noise from
conductive polymers when used as supporting matrix for probe attachment or
entrapment for biomolecule detection.

30 It is still another object of the present invention to provide a simplified method
for biomolecule detection and a biosensing device to carry-out such detection.

35 The invention is directed to a method of detecting biological molecule
(biomolccule) such as DNA, RNA and polypeptides with the aid of a neutralized
conjugated polymer or copolymer on electrodes. Compared to prior art, the present
40 invention makes use of a functionalized polymer or copolymer in its neutral state,
instead of conductive state as the supporting matrix for biomolecule probe attachment
25 or entrapment in a biomolecule detection device.

45 In one embodiment of the invention, aromatic monomers and functionalized
aromatic monomers are electrochemically polymerized and deposited on an electrode
surface to generate a functionalized polymer or copolymer. The as-deposited
50 conjugated polymer or copolymer is in a charged, conductive state. In present

5 invention, the charged, functionalized polymer or copolymer is electrochemically reduced to a neutral state to form (charge neutral conjugated polymer) before it is used in any biomolecule detection.

10 The charge neutral functionalized polymer or copolymer has low electric background when used in electric detection of biomolecules. It also does not quench fluorescent signal when used in fluorescent detection of biomolecules. In both cases, the resulting devices have significantly improved signal to noise ratio, thus enhancing the sensitivity of biomolecule detection.

20 Thus, the invention includes a charge neutral conjugated polymer which have functional groups for binding biomolecule probes to the polymer. The invention includes electrodes in electrical communication with such polymers, arrays of such electrodes. The invention includes biosensors which a biomolecule probe is covalently linked to the functional group of the charge neutral conjugated polymer on electrode and a binding of a biomolecule to be detected is measured by an electrical detection means, such as AC impedance.

20 BRIEF DESCRIPTION OF THE DRAWINGS

35 Figure 1 represents a schematic diagram for preparing the array of polypyrrole coated electrodes and detecting by AC impedance.

40 Figure 2 illustrates polypyrrole copolymer formulation.

Figure 3 illustrates the electrochemically reduced neutral polypyrrole copolymer.

45 Figure 4 illustrates the relationship of capacitance vs. frequency on oxidized polypyrrole-based electrodes with and without DNA Attachment.

50 Figure 5 illustrates the relationship of capacitance vs. frequency on neutral polypyrrole-based electrode with and without DNA attachment.

Figure 6 illustrates the comparison of response of capacitance vs. frequency between oxidized and neutralized polypyrrole-based electrodes with DNA attachment.

Figure 7 AC impedance planes measured in perfect match hybridized DNA and single stranded DNA system.

Figure 8 is a Frequency Complex diagram obtained from neutralized polypyrrole Electrodes.

Figure 9 is impedance planes measured in 3-bas mismatch hybridized DNA and single stranded DNA systems.

Figure 10 is a plot of Resistance vs. $\omega^{-1/2}$ for AC impedance measured in 3-base mismatch hybridized DNA and single stranded DNA systems.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to a method of detecting biological molecule with the aid of a charge neutral conjugated polymer on electrodes. Charge neutral conjugated polymer is meant a polymer with zero charge (negative or positive) on its backbone, yet with delocalized pi electron on its backbone. A conjugated polymer is characterized by its backbone with regular alternation of single and double chemical bonds. Examples of conjugated polymers include: polypyrrole, polyphenylene, polyacetylene, polydiacetylene, polythiophene, polyfuran, polyaniline, polycarbazole, poly(phenylene vinylene). More specifically, the invention encompasses a charge neutral conjugated polymers containing one or more functional groups capable of binding a probe molecule. The charge neutral conjugated polymer deposited on the surface of electrodes by electrochemical copolymerization of aromatic monomers and functionalized monomers as is known in the art. The as-deposited conjugated polymer or copolymer is conductive and is usually in its charged state with its charge

5 being balanced by counter ions from the polymerization solution. The charged state is the source of signal quenching for nearby fluorescent markers as in the case of fluorescence detection. It is also the source of noise for electric detection.

10 To overcome these potential problems, the polymer or copolymer deposited on the electrode used in present invention is reduced to its charge-neutral state from the as-deposited charged state by reverse biasing right after the polymer or copolymer is initially deposited on the surface electrodes. The polymer or copolymer in its neutral state is an insulator or semiconductor, which does not quench fluorescence of nearby fluorescent markers in fluorescence detection and also give rise to only limited background noise in electric detection of biomolecule target.

15 The functional group used in present invention includes, but not limited to, amine, hydrazine, ester, amide, carboxylate, halide, hydroxyl, vinyl, vinyl carboxylate, thiol, phosphate, silicon containing organic compounds, and their derivatives. The functional group is used to bind biomolecule probes such as DNA, RNA, peptides, polypeptides, proteins, antibody, antigen and hormones to the polymer or copolymer on the electrode. For example, an oligonucleotide which is in part complementary to a target DNA is covalently linked to a neutral polypyrrol copolymer through an amine functional group.

20 The electrode used in the present invention is made of at least one of the following materials: metals such as gold, silver, platinum, copper, and alloys; conductive metal oxide such as indium oxide, indium-tin oxide, zinc oxide; other conductive materials such carbon black, conductive epoxy and combinations thereof.

25 The preferred sensing method in this embodiment is electric or electrochemical methods. After exposure to a target molecule, the biosensor senses a change in electric signal, and reports the change by a readout means such as display,

5 5 printout. The electric or/and electrochemical methods may be selected from, but are
not limited to, AC impedance, cyclic voltammetry (CV), pulse voltammetry, square
10 10 wave voltammetry, AC voltammetry (ACV), hydrodynamic modulation voltammetry,
potential step method, potentiometric measurements, amperometric measurements,
current step method, and combinations thereof.

15 10 It is more advantageous to detect a biomolecule target without the need of
labeling the target. Present invention provides a highly sensitive method for detection
of biomolecule target without the need of labeling the target.

20 20 Some biomolecules are electrically active and may produce undesired
background noise when a detection is performed by passing charge through those
15 15 biomolecules. For example, guanine and adenine can be oxidized around 0.75 V and
25 25 1.05 V, respectively. (Analytica Chimica Acta 319 (1996) 347-352). Thus it is more
desirable to use impedance methods for labelless biomolecule detection.

30 30 The invention includes a method for determining an analyte in a test sample
comprising:

- 20 20 (a) depositing a polymer or copolymer film on an electrode by
35 35 electrochemically polymerizing an aromatic monomer and a
monomer with functional group in a solution via a positive bias
with supporting electrolyte;
40 40 (b) neutralizing the polymerized polymer by applying a reverse
25 25 bias to the electrode;
45 45 (c) attaching covalently a biomolecule probe to the neutral
copolymer through the functional monomer;
50 50 (d) contacting the electrode with the test sample containing an
electrolyte; and

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5 5 In a variation of the aforesaid embodiment, the biomolecule probe can also be
attached to the aromatic functional monomer before it is electrochemically
polymerized with aromatic monomer to yield a conjugated polymer.

10 This invention will be further described by the following example with
polypyrrole as the conjugated copolymer and DNA as detection target. The example
15 10 is intended to illustrate specific embodiments of the invention but not to limit this
invention in spirit or scope.

Example 1

20 In order to demonstrate this invention, platinum electrodes with a diameter of
2 mm were used for electrochemical deposition of polypyrrole. The electrode surface
15 was polished by gamma alumina powder (CH Instruments, Inc.) with 0.3 and 0.005
25 μm in sequence followed by deionized water washing. After polishing, the electrodes
were immersed in 1 M H₂SO₄ for 20 minutes and then vigorously washed by DI
30 water. CH 660 potentiostat was used for polypyrrole deposition. Platinum wire and
Ag/AgCl were used as the counter electrode and reference electrode, respectively. A
20 solution containing 0.1 M pyrrole + 5 μM 3-acetate N-hydroxysuccinimido pyrrole +
35 0.1 M LiClO₄/acetonitrile (0.5% water) was prepared as the electrolyte. Cyclic
voltammetry (CV) was used for the electrochemical deposition. The potential range
for the CV was 0.2-1.3 V vs Ag/AgCl for the first cycle and then changed to -0.1 to
40 1.0 vs Ag/AgCl for other five cycles. An electrochemical oxidation of the pyrrole
25 produced polypyrrole as shown in Figure 2.

45 The electrolyte was purged by nitrogen gas during whole electrochemical
deposition. The deposited polypyrrole film with the linking function group was
uniform and blue in color. The polypyrrole film is in oxidized form (charged
50 conductive state). To make a neutralized polypyrrole, the electrode was placed in the

5 electrolyte again and cycled over a potential range of -0.2 to 0.3 vs Ag/AgCl, which
is the reduction zone for this electrochemical system. The neutralization of the
polypyrrole film is illustrated in Figure 3. The neutralized polypyrrole film coated
10 electrodes were vigorously washed for probe oligonucleotide attachment.

Then a 5'-amino-substituted oligonucleotide was attached onto the neutral
15 polypyrrole film by a direct substitution of the leaving N-hydroxysuccinimide group
in dimethylformamide containing 10% phosphate buffer at pH = 8.0 at room
temperature for 16 hours. The oligonucleotide CCC TCA AGC AGA with a terminal
20 amino group on its 5'-phosphorylated position was used. For comparison, the
oxidized polypyrrole film was modified by oligonucleotide in the same procedure
15 mentioned above.

Oxidized and neutralized polypyrrole deposited electrodes with and without
DNA attachment were tested in deionized water by Solartron 1260 impedance
30 analyzer. A platinum sheet with area of 10 cm² was used as the counter electrode.
Frequency sweeping method with a bias of 500 mV was conducted over frequency
20 range of 100 mHz to 1 MHz. Since the double layer capacitance is proportional to the
area of the electrode surface, the capacitance of the counter electrode surface, $C_c \gg C_p$.
35 C_p represents the probe electrode capacitance. Thus, the total capacitance of the
detecting system $C_t = 1/(1/C_p + 1/C_c) = C_p C_c / (C_p + C_c) = C_p$. In addition, the solution
40 resistance for a disc-shaped ultramicroelectrode can be expressed as:

$$25 \quad R_u = 1/(4kr) \quad (1)$$

45 Where r is the radius or the side length of the electrode and k is the
conductivity of the solution. The R_u contributed from the small probe is much larger
than the counter electrode. The results obtained from the AC impedance can

5 represent the change from the probes, since the surface area of the probe is much smaller than that of the counter electrode.

10 Experimental results are shown in Fig. 4, 5 and 6. Fig. 4 shows the capacitance changes of the electrode surface vs. frequency, indicating that the oxidized polypyrrole-based electrode surface with oligonucleotide attachment has
15 larger capacitance response than the surface without oligonucleotide attachment at the low frequency range. However, the ratio of signal to noise is not great. Fig. 5 demonstrates that the capacitance of neutralized polypyrrole-based electrode surface with oligonucleotide attachment is significantly greater than that of the surface without oligonucleotide attachment. Fig. 6 shows that the capacitance on the
20 neutralized polypyrrol-based electrode surface with oligonucleotide attachment is greater than that of oxidized polypyrrole-based surface by about 4 times.

The hybridization of the oligonucleotide probe on neutral polypyrrole with its complementary strand shows significant improved signal to noise ratio as compared to that on charged polypyrrole.

20 Example 2

35 The neutralized polypyrrole film coated electrodes were vigorously washed for DNA attachment. The electrodes coated with polypyrrole were placed in a mixture of 80 μ L of DMF and 20 μ L of 15 nM of 5i-amino-3i-fluorescein labeled 15 bp
40 oligonucleotide for 4 hrs at room temperature. At the end the electrode was washed with TBE buffer, deionized water thoroughly, and dried at room temperature in the air. The condition is not optimized.

45 The 5'- amino-substituted oligonucleotide of 300 uM concentration in 25 uL of dimethylformamide containing 20% phosphate buffer at pH = 8.0 was attached onto
50 the neutral polypyrrole film on a microelectrode by a direct substitution of the leaving

5 N-hydroxysuccinimide group at room temperature for 16 hours. The
oligonucleotide CCC TCA AGC AGA with a terminal amino group on its 5'-
phosphorylated position was used as an example. After the reaction, the
10 microelectrode was washed with DI water thoroughly before a baseline AC
impedance was measured. For hybridization, the probe attached to polypyrrole on a
15 microelectrode was exposed to 35 μ L of target molecule of different concentration
(μ M to aM) in 1x SSC buffer. The hybridization takes place in a sealed conical tube
at 37 C in a water bath for 24 to 48 hrs. The microelectrode was then washed with
20 ample amount of 1x SSC solution at room temperature before AC impedance
measurement.

25 A Solartron Impedance Frequency Analyzer 1260 with Electrochemical Interface
1287 was used to measure the impedance before and after hybridization of the
polypyrrole microelectrodes. The counter and reference electrodes were platinum and
30 Ag/AgCl, respectively. The measurements were conducted at open circuit voltage
(OCV) in 1 M LiClO₄ solution. The measured complex impedance versus frequency
20 is shown in Fig. 8 for single and hybridized DNA, indicating significant difference of
35 the impedance before and after hybridization.

In this experiment, this type of electrodes can detect 0.1 amol of target DNA in
40 solution due to the neutralized form of polypyrrole film.

25 Example 3

45 Experiments for the specificity of the polypyrrole based electrodes were
conducted. Eight probes attached electrodes were hybridized in buffers containing
2pM and 2 fM of perfectly matched and three base mismatched target 15mer DNAs,
50 respectively. Results show significant difference between perfect and mismatched

5 hybridized DNA. Further, the electrodes were placed in 1XSSC buffer for 30 min. of
washing at 37 and 38°C, respectively. AC impedance measurements demonstrate that
10 the AC impedance for the mismatched hybridization was getting closer and closer to
the baseline of the single stranded DNA with the increase of the washing temperature
while that for the perfectly matched hybridization was almost keeping constant. The
15 results are shown in Fig. 7 and 8. Fig. 9 is plotted from Fig. 8, indicating that the
resistance in the mismatched DNA system continuously decreases with the increase
of the washing temperature going back to the baseline of the single stranded DNA.

20 This invention can be used in any solution containing metal or polymerized
cations, which are ion-conductive and can react with DNA.

15 25 The above examples are intended to illustrate the present invention and not to
limit it in spirit or scope.

Claims

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5 What is claimed is:

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1. A charge neutral conjugated polymer having functional groups for binding a biomolecule probe.

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2. A charged neutral conjugated polymer of claim 1 with a functional group for binding a biomolecule probe to the charge neutral conjugated polymer wherein the charge neutral conjugated polymer is selected from the group consisting of polypyrrole, polyphenylene, polyacetylene, polydiacetylene, polythiophene, polyfuran, polyaniline, polycarbazole, poly(phenylene vinylene) and copolymers and combinations thereof.

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3. A polymer of claim 1 wherein the polymer is prepared by electrochemical polymerization.

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4. A polymer of claim 1, wherein the functional group is selected from the group consisting of amine, hydrazine, ester, amide, carboxylate, halide, hydroxyl, vinyl, vinyl carboxylate, thiol, phosphate and silicon containing organic groups.

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5. An electrode in electrical communication with the charge neutral conjugated polymer of Claim 1.

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6. An electrode of claim 5 wherein the electrode comprises gold, silver, platinum, copper, and alloys, indium oxide, indium-tin oxide, zinc oxide; or carbon black, conductive epoxy, or combinations thereof.

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7. An array of electrodes of Claim 6.

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8. The array of electrodes of claim 7 wherein the charge neutral conjugated polymer is polypyrrole.

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9. A biosensor device for detecting a biomolecule comprising a an electrode which is in electrical communication with a matrix of charge neutral conjugated polymer having a functional group wherein a biomolecule probe is covalently linked to the functional group and a means for electrically detecting the binding of the biomolecule to the biomolecule probe.

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10. A biosensor for detecting a biomolecule comprising an array of electrodes which are in electrical communication with a matrix of charge neutral conjugated polymer having a functional group wherein a biomolecule probe is covalently linked to the functional group and a means for electrically detecting the binding of the biomolecule to the biomolecule probe.

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11. The biosensor of claim 10 wherein the electrical detection means is selected from AC impedance, cyclic voltammetry (CV), pulse voltammetry, square wave voltammetry, AC voltammetry (ACV), hydrodynamic modulation voltammetry,

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5 potential step method, potential step method, potentiometric measurements,
amperometric measurements, current step method, and combinations thereof.

10 12. The biosensor of claim 11 wherein the electrical detecting means is AC
impedance.

15 13. The biosensor of claim 12 wherein one or more of the electrodes have
an oligonucleotide which is partly complementary to a target DNA covalently linked
20 to the charge neutral conjugated polymer in electrical communication with the
electrodes.

25 14. The biosensor of claim 13 wherein DAN in a test sample is hybridized
to the oligonucleotide covalently linked to the charge neutral conjugated polymer on
30 one or more of the electrodes in the array.

20 15. A method for determining an analyte in a test sample comprising:

35 (a) providing an electrode which is in electrical communication with a
matrix of charge neutral conjugated polymer which has covalently linked a binding
group which directly or indirectly binds to the analyte;

40 (b) contacting the matrix of charge neutral conjugated polymer with the
25 test sample containing the analyte; and

45 (c) electrically detecting the analyte bound to the neutral conjugated
polymer.

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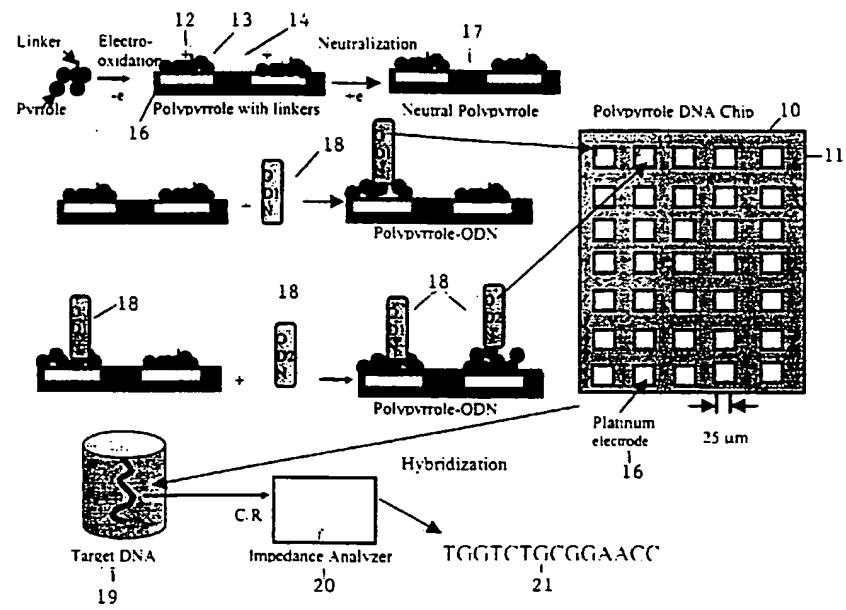


FIGURE 1

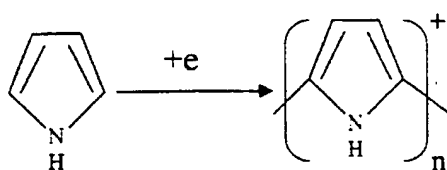


FIGURE 2

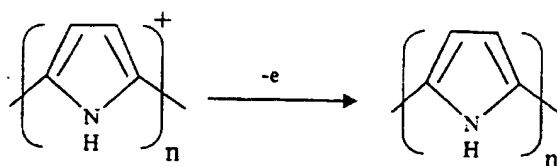


FIGURE 3

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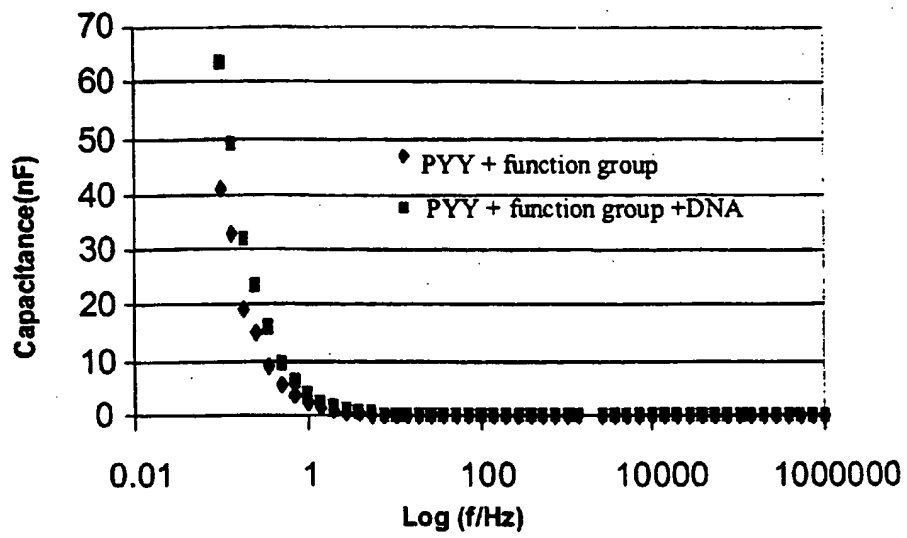


FIGURE 4

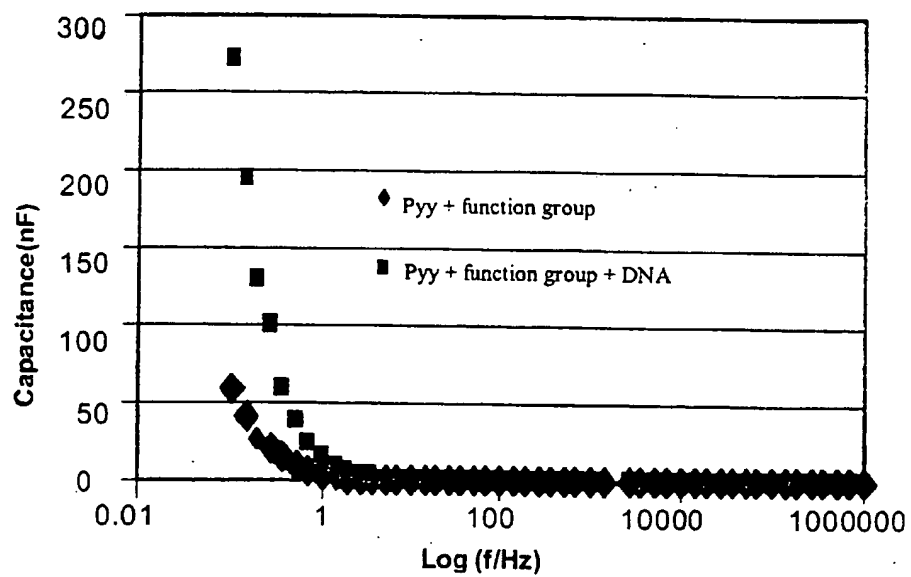


FIGURE 5

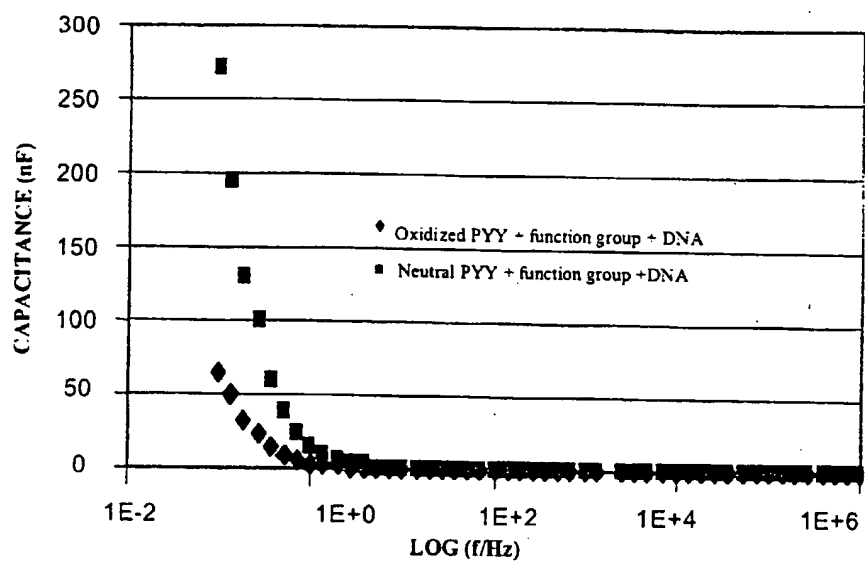
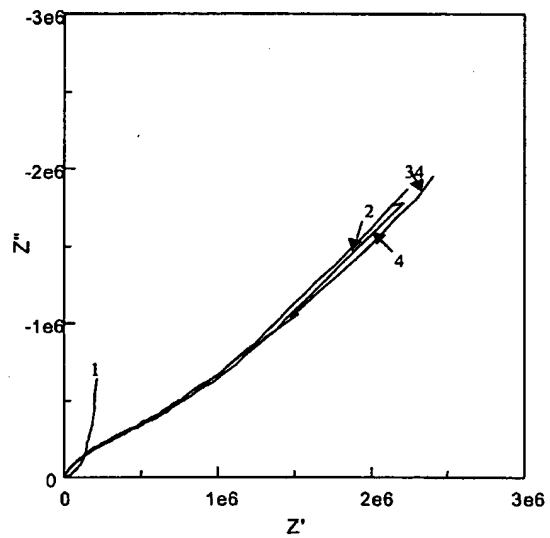


FIGURE 6



1. Single stranded DNA (Probe)
2. Hybridized DNA with perfect match in 30 μ l of 2 pM target for 48 hrs
3. After washing 2 at 37 C for 30 min.
4. After washing 3 at 38 C for 30 min.

Fig. 7 AC impedance planes measured in perfect match hybridized DNA and single stranded DNA system

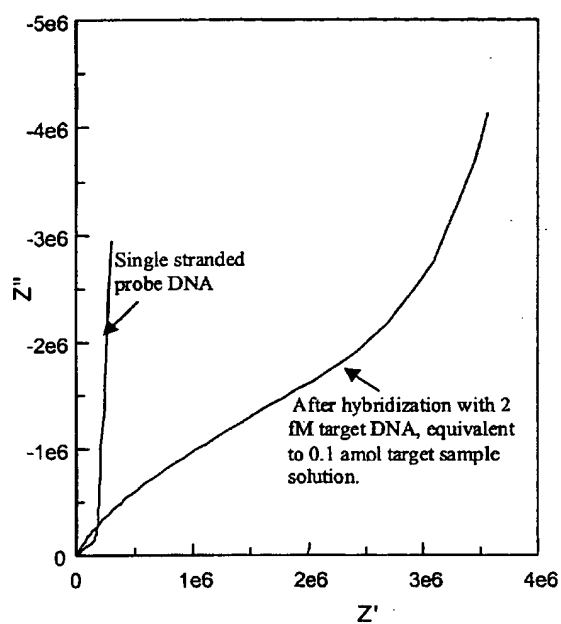


Fig. 8 Frequency Complex diagram obtained from neutralized polypyrrole Electrodes

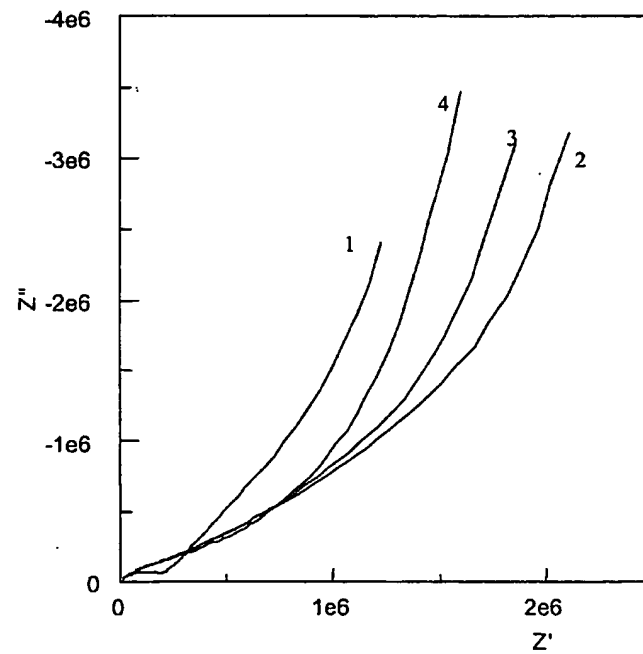


Fig. 9 AC impedance planes measured in 3-base mismatch hybridized DNA and single stranded DNA systems

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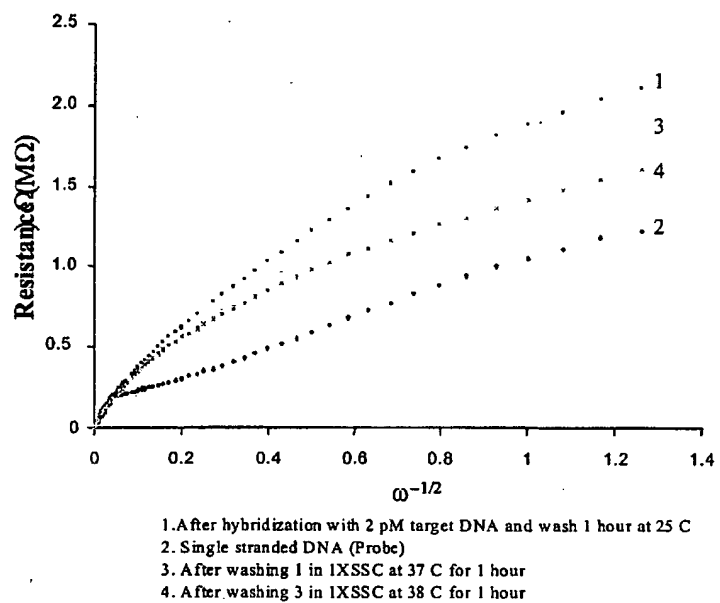


Fig. 10 Plot of Resistance vs. $\omega^{-1/2}$ for AC Impedance measured in 3-base mismatch hybridized DNA and single stranded DNA systems

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/15832

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 601N33/543 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 601N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GARNER B; GEORGEVICH A; HODGSON A J; LIU L; WALLACE G G: "Polypyrrole-heparin composites as stimulus-responsive substrates for endothelial cell growth" JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, vol. 44, February 1999 (1999-02), pages 121-129, XP000964695 the whole document	1-6
A	SARGENT, ANITA ET AL: "The electrochemistry of antibody-modified conducting polymer electrodes" J. ELECTROANAL. CHEM. (1999), 470(2), 144-156 XP000964567 the whole document	1-17
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

15 November 2000

Date of mailing of the international search report

24/11/2000

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Hart-Davis, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/15832

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Information on patent family members

International Application No

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